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RESPONSES OF *CHAETOPTERUS VARIOPEDATUS* TO OSMOTIC STRESS, WITH A DISCUSSION OF THE MECHANISM OF ISOOSMOTIC VOLUME-REGULATION

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Detailed studies on salt and water balance in polychaetes have been primarily directed toward free-living intertidal and estuarine species (see Olgesby, 1969a and 1969b, for extensive reviews). Less information is available on sedentary tube-dwelling worms—particularly those which appear to be stenohaline. One such species, *Chaetopterus variopedatus*, occurs widely along coastal areas in the intertidal to subtidal zones. Although it is usually found in regions of stable high salinity, it has been reported from areas where the salt concentration reaches as low as 20‰ (Gosner, 1971). In addition, *Chaetopterus* is known to occur intertidally in areas of heavy seasonal rainfall which may be presumed to produce some dilution of the interstitial seawater. It has been pointed out, however, that the actual salinities immediately surrounding burrowing or tube-dwelling organisms may be considerably different than a cursory sampling of the surrounding water would suggest (Gunter, 1961; Oglesby, 1969b). It is of interest in this connection that Garrey (1905) could find no evidence that *Chaetopterus* could regulate its salt or water content when subjected to salinities below that of approximately 31‰. The present investigation was undertaken, therefore, to examine in greater detail the responses of *Chaetopterus* to osmotic stress.

Materials and Methods

Source and maintenance of animals

Specimens of *Chaetopterus* were obtained from Pacific Bio-Marine Supply Co., Venice, California. In the laboratory, the animals (within their natural tubes) were maintained at 16° C in aerated recirculating aquaria containing 50 gallons of artificial seawater ("Instant Ocean," Aquarium Systems, Inc., Cleveland, Ohio). Worms were fed *ad libitum* daily with "Fryfare" extra fine fish food (Wardley Products Company, Inc., Long Island City, New York). Animals were acclimated for a minimum of 7 days under these conditions before any experiments were performed.

Preparation of test solutions

Hypoosmotic solutions were prepared by diluting the artificial seawater in which the worms had been maintained with an appropriate amount of distilled water.

Weight determinations

For the determination of wet weights, the animals were blotted dry with absorbent paper towels and weighed in air (at 16° C) to the nearest milligram on a torsion balance (The Torsion Balance Co., Clifton, New Jersey).

Collection of coelomic fluid samples

Prior to removal of coelomic fluid, the animals were thoroughly blotted with absorbent paper towels. With the aid of a binocular dissecting microscope, the tip of a 200 μl capillary tube, drawn out to a fine point, was inserted through the integument into the large coelomic cavity of one (or more) of the "fan" segments. Fluid was then drawn into the capillary with the aid of a mouth suction-tube. Depending on the size of the animal, from 50 to 150 μl of fluid could be obtained from a single segment in this fashion. A final sample from each worm (in excess of 125 μl) was obtained by pooling the coelomic fluid from the three fan segments, as needed. The capillary tubes were flame-sealed and stored at −20° C. Since coelomic fluid from these segments was virtually devoid of cells, the samples were not centrifuged prior to making determinations.

Determination of osmotic pressure, Na^+ and Cl^-

Osmotic pressure was measured with an Advanced High Precision freezing-point osmometer (Advanced Instruments, Inc., Newton Highlands, Massachusetts) in the following manner. Duplicate 50 μl samples of coelomic fluid were diluted to 200 μl with glass-distilled water and placed in sample holders (0.2 ml) for determination. The final results were calculated by comparison to a standard curve made from solutions of known osmolarity (standard solutions from Advanced Instruments, Inc.) with correction for electrolyte activity change caused by dilution.

For sodium determinations, duplicate 5 μl samples of coelomic fluid were diluted to 5.00 ml with glass-distilled water. Flame photometry was carried out using a Coleman Jr. II spectrophotometer with flame attachment, and the results compared to a standard curve made from solutions of known sodium concentration.

For chloride determinations, duplicate 5 μl samples of coelomic fluid were diluted to 50 μl with glass-distilled water. Titrimetric determinations were made using an Oxford automatic titrator (Oxford Laboratories, San Mateo, California) with acid mercuric nitrate in the presence of s-diphenylcarbazone as an endpoint indicator. Results were compared to standard solutions of known chloride concentration.

Results

Long-term adaptation to hypoosmotic seawater

To examine the response to long-term, gradual lowering of salinity in the external medium, 50 worms (in their natural tubes) were allowed to acclimate for one week in artificial seawater of 1090 milliosmoles concentration. The health of the worms was estimated by cutting off one of the tips of the "U"-shaped tubes and observing whether or not the tip was repaired. Rapid (overnight) repair of its tube house was taken as a sign that a worm was in good condition. Five

worms were removed from their tubes and coelomic fluid and medium samples taken. The salinity in the acclimation aquarium was then gradually lowered at a rate of approximately 3% per day by addition of calculated amounts (volumetric) of distilled water. At varying intervals, groups of six worms in their tubes were transferred to other aquaria and kept for seven days at the lowered salinity attained. All worms were fed daily. At the end of the week period, worms were removed from their tubes, examined, and coelomic fluid and medium samples taken. The data for total osmolarity, sodium, and chloride concentrations are shown in Figures 1–3.

Over the range studied, it appears that the coelomic fluid of *Chaetopterus* conforms to the external medium with respect to total osmolarity and sodium and

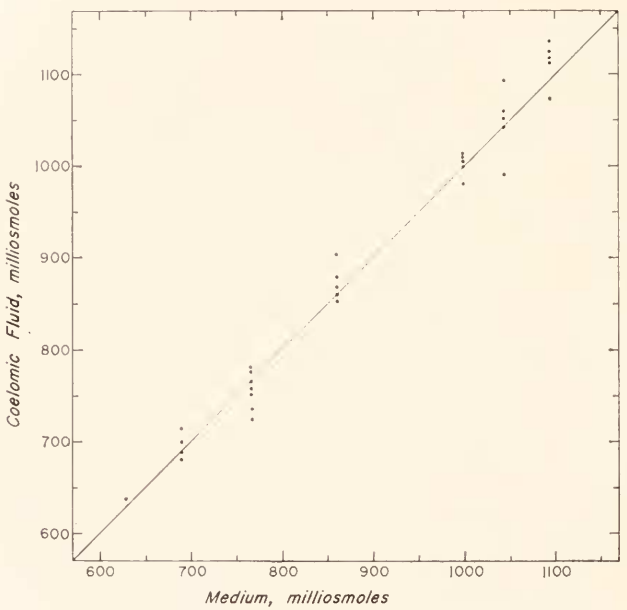

FIGURE 1. The osmotic concentration of *Chaetopterus* coelomic fluid in relation to that of the external medium, all points shown.

chloride concentration. There is no indication of ionic or osmotic regulation for the parameters examined. Only a single animal (out of 10) survived the slow dilution and week-long maintenance at 630 milliosmoles (*i.e.*, 57.8% of the initial value). It would appear then, that approximately 55–60% seawater is the lower limit which *Chaetopterus* can tolerate for moderately extended periods of time. Survivorship and/or regulation was not examined in hyperosmotic solutions exceeding *ca.* 1100 milliosmoles.

Volume changes during osmotic stress

To examine the effects of osmotic stress on water and salt movements, worms were carefully removed from their tubes and placed individually in 4-inch finger

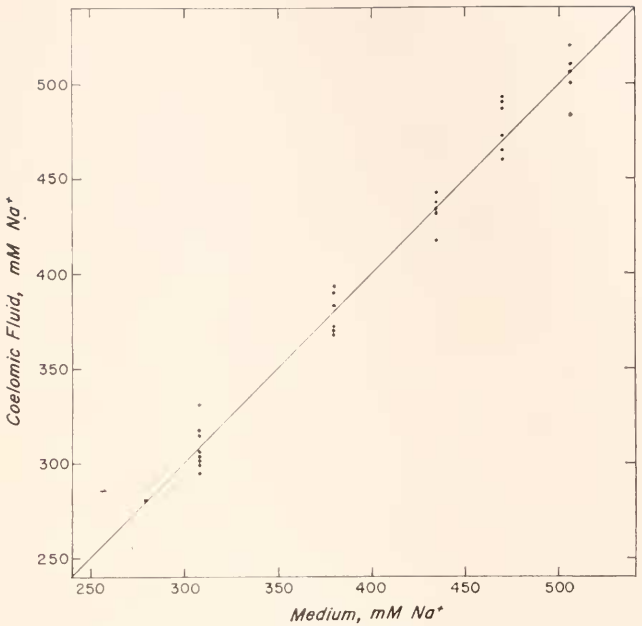

FIGURE 2. The sodium concentration of *Chaetopterus* coelomic fluid in relation to that of the external medium, all points shown.

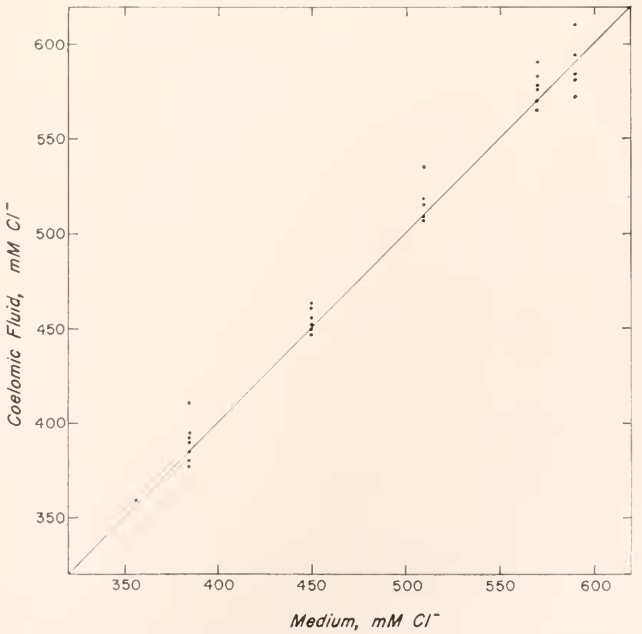

FIGURE 3. The chloride concentration of *Chaetopterus* coelomic fluid in relation to that of the external medium, all points shown.

bowls filled with 200 ml of artificial seawater. They were then carefully examined, and any appearing unhealthy, injured, or incapable of producing the characteristic rhythmic pumping activity were discarded. Stage of maturity and sex were recorded for each of the experimental animals. The worms were distributed by size so that each group (experimentals and control) had a representative size distribution. Initial weights were taken and experimental animals were placed in hypoosmotic test solutions of known salinity. All individuals were then weighed at successive 30-min intervals for a period of six hours, or until they were removed from the experiment because of rupture (group A). After six hours had

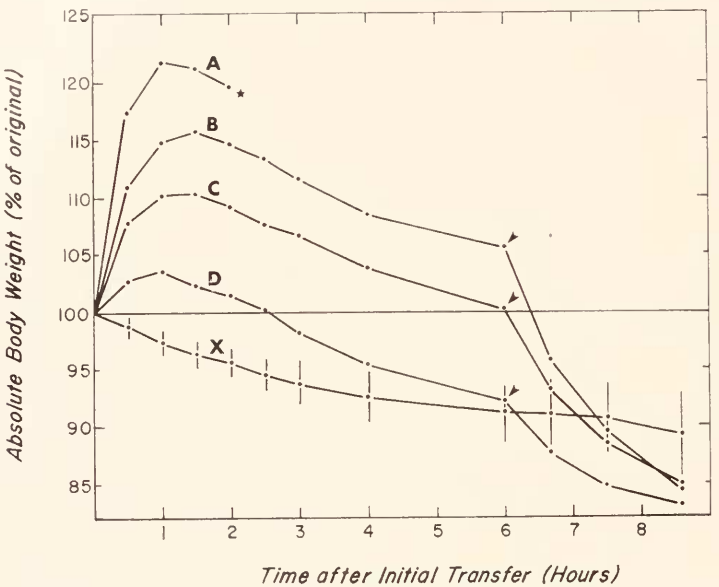

FIGURE 4. Time-course of weight changes in *Chaetopterus* after direct transfers from 901.4 milliosmoles to: (A) 451.4 milliosmoles (n = 5); (B) 681.8 milliosmoles (n = 7); (c) 749.6 milliosmoles (n = 8); (D) 802.8 milliosmoles (n = 10); (X) controls, kept at 901.4 milliosmoles (n = 11). Star on curve (A) indicates all animals had ruptured, and were removed from experiment. Arrows at 6 hours indicate transfer back to initial medium. Vertical bars represent one S.E. above and below the mean, and are indicated for one curve only, for clarity, as representative of the amount of variability encountered in all curves.

elapsed, the worms were transferred back to seawater of the initial strength and weight monitored for an additional $2\frac{1}{2}$ hours as before. The data are shown in Figure 4 (as absolute per cent body weight change) and Figure 5 (corrected for controls). Typical variability is indicated by the mean ± 1 standard error.

The data in Figures 4 and 5 show that: (1) control worms (remaining under acclimated osmotic conditions) slowly lose weight, presumably due to handling; (2) worms subjected to hypoosmotic solutions rapidly gain weight, roughly in proportion to the degree of osmotic stress; (3) weight gain is essentially complete in 1.5 hours, and is followed by a period of both absolute and relative weight loss; and (4) following transfer back to initial salinity, all experimental groups rapidly

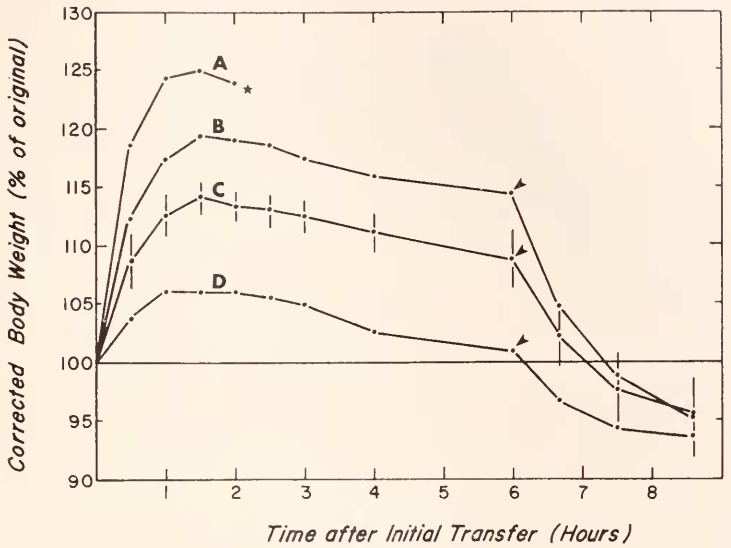

FIGURE 5. As in Figure 4, corrected for controls.

lose weight, ultimately dropping below control group values. No significant differences were found with regard to sex or state of maturity.

To examine further the effects of sudden osmotic stress on weight and coelomic fluid constituents, 30 animals were removed from their tubes, examined, weighed and assigned to control (10) and experimental (20) groups as before. The experimental animals were transferred to a hypoosmotic solution (*ca.* 80% acclimated seawater) at timed intervals. At 1.5 hours elapsed time all animals were weighed, and coelomic fluid samples from five control and five experimental

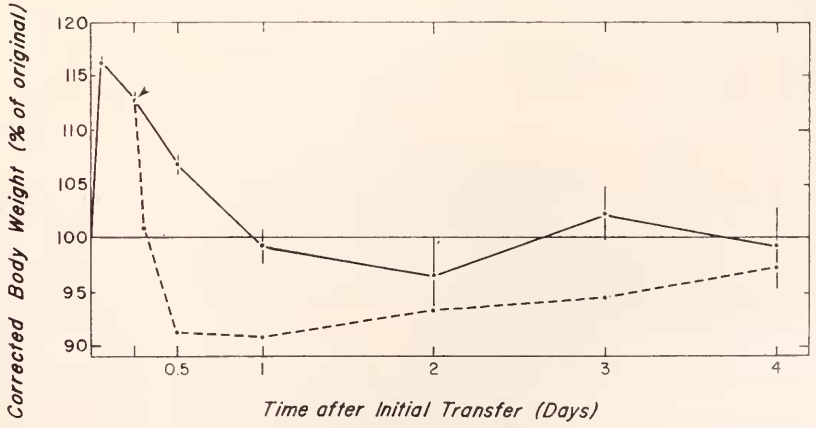

FIGURE 6. Time-course of weight changes (corrected for controls) in *Chaetopterus* after direct transfer from 1100 to 875 milliosmoles. Arrow indicates transfer back to initial medium for one group (broken line). For clarity, ±1 standard error shown for one group only; n = 5 for all points after 6 hours.

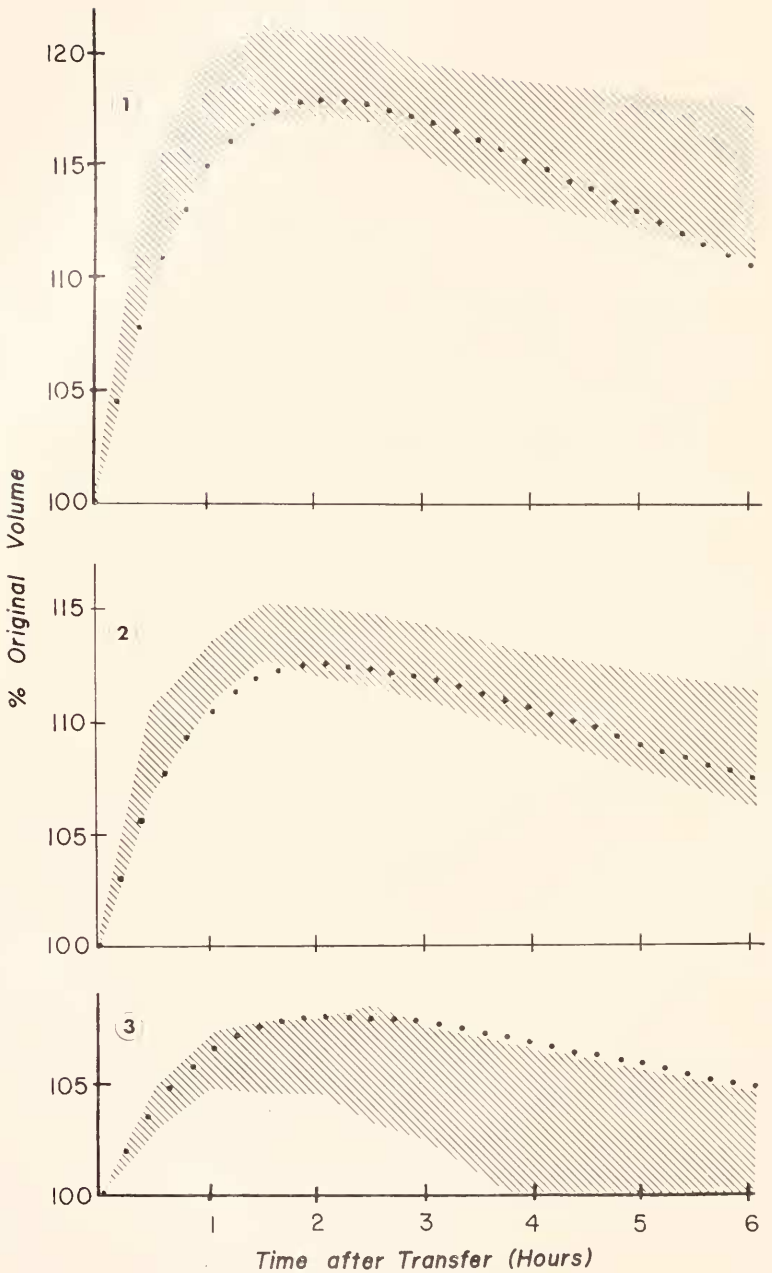

FIGURE 7. Experimental results *versus* behavior predicted from theoretical model. Cross-hatch indicates experimental data (mean ± 1 standard error); dotted line from computer solution to equations; (1) $\Delta C = 0.76, \beta = -0.30$; (2) $\Delta C = 0.83, \beta = -0.30$; (3) $\Delta C = 0.89, \beta = -0.30$; (4) $\Delta C = 0.76, \beta = -0.29$; (5) $\Delta C = 0.83, \beta = -0.56$; (6) $\Delta C = 0.89, \beta = -0.17$.

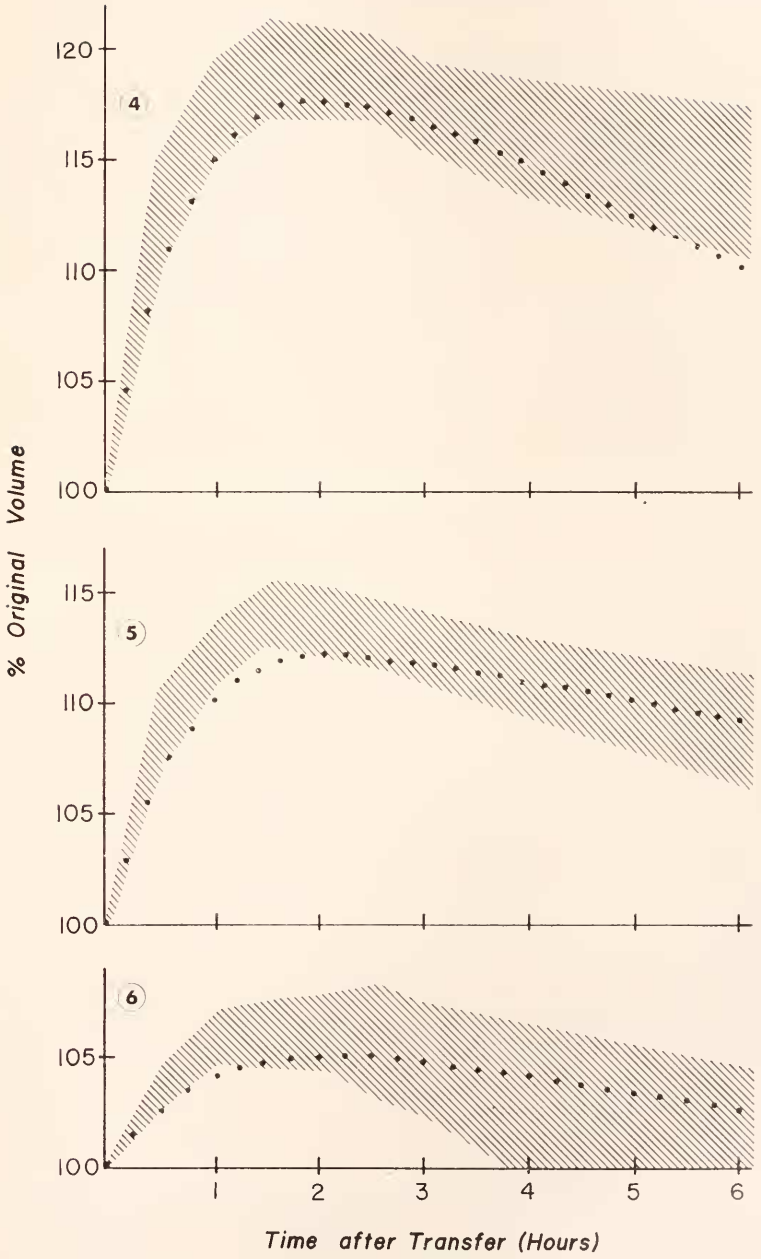

FIGURE 7—(*Continued*)

animals (as well as medium samples) were taken. At six hours all remaining animals were weighed, and ten experimental animals transferred back to initial salinity conditions. One and one-half hours after transfer, five animals were removed from the latter group, and coelomic fluid and medium samples taken. The weight of the remaining 15 animals (five per group) was monitored at regular intervals over a period of four days. The worms were provided with food and substitute "houses" (consisting of glass tubes of appropriate diameter) following the 12th-hour weighing. Clean water of the proper salinity was added daily. Data on the weight changes of the experimental groups (corrected for controls) are shown in Figure 6. The results indicate that animals subjected to sublethal hypoosmotic stress return to approximately initial weight values after about 24 hours (Fig. 6, solid curve). In view of the low number of animals involved and the variability exhibited, no significance is ascribed to the apparent oscillatory behavior of the mean weight around the 100% value. Those animals transferred back to the initial salinity lose weight rapidly, ultimately dropping to *ca.* 90% of their starting value (Fig. 6, broken curve). There follows a slow climb in weight toward the starting values.

Coelomic fluid: medium ratios for osmolarity, sodium and chloride, taken 90 minutes after transfer to new media, are given in Table I. The data show the coelomic fluid to be not significantly different from the medium with respect to osmotic and ionic concentration.

Discussion

Data from the present investigation suggest that *Chaetopterus variopedatus* is an ionic- (Na^+; Cl^-) and osmoconformer at seawater concentrations from 1100 milliosmoles down to a lethal lower limit of approximately 630 milliosmoles. This corresponds to a range of 116%–66% seawater (with "100%" seawater = 950 milliosmoles). These data are consistent with measurements for other marine polychaetes, which indicate that the majority of these annelids are osmoconformers at the higher range of salinities (Oglesby, 1969a). The fact that total osmotic pressure closely parallels the sodium and chloride concentrations indicates that there is only minor contribution (if any) from soluble organic molecules to the total osmotic concentration of the body fluids, even at lowered salinities. Little is known directly about the organic constitutents of *Chaetopterus* body fluids, except that there is no dissolved respiratory pigment (Dales, 1969) and that uric acid occurs at a concentration of 43.4 μMoles/l (Wilber, 1948). It is concluded that the major osmotic characteristics observed result almost exclusively from water and inorganic salt fluxes.

Table I.

Coelomic fluid: medium ratios 1.5 hours after transfer to new medium, mean ±1 standard error; n = 5 for all values

Following transfer:	mOsm (CF:M)	Na^+ (CF:M)	Cl^- (CF:M)
From 1100 to 875 mOsm	1.02 ± 0.029	0.99 ± 0.066	1.06 ± 0.067
From 875 to 1100 mOsm	0.99 ± 0.016	1.01 ± 0.019	0.99 ± 0.011

The rapid increase in weight (volume) of worms directly transferred to hypoosmotic media presumably reflects an osmotically driven influx of water through a readily permeable integument. The volume increase reaches a maximum in 1.5 hours (Figs. 4, 5, and 6) at which time the coelomic fluid has again reached osmotic equilibrium with the medium (Table I). Comparison of initial slopes of weight gain (after initial transfer) with weight loss (after back-transfer) also clearly indicates that, under the same osmotic gradient, fluid enters more rapidly than it leaves. This apparent greater permeability inward than outward has been reported for other species by a number of investigators (Adolph, 1936; Gross, 1954; Jørgensen and Dales, 1957; Oglesby, 1965) although the reason for it is not known.

That some salt efflux has also occurred is indicated from the results of experiments where hypoosmotically stressed worms were returned to initial salinities (Figs. 4, 5, and 6). In every case, worms rapidly lost weight and eventually dropped well below the mean weight of the control groups. Weight loss is essentially complete after $1\frac{1}{2}$–2 hours (Figs. 4, 5, and 6), after which there appears to be a slow climb toward initial values (Fig. 6). Shrinkage to less-than-original volume after transfer back to normal seawater shows that the equilibrium attained in dilute seawater was reached partly by the loss of solutes (Potts and Parry, 1963),

A return to initial weight (volume) values after transfer to media of various salinities is conventionally used as the criterion for judging whether or not a marine animal has the ability to control its body volume (Oglesby, 1969a). Applying this criterion to data on the time-course of weight changes in hypoosmotically stressed *Chaetopterus* (Figs. 4, 5, and 6), one must conclude that *Chatetopterus* possesses volume-regulating ability over most, if not all, of its viable salinity range. Garrey (1905) is apparently the only previous investigator to publish data on the response of *Chaetopterus* to hypoosmotic media. He transferred worms directly from full-strength Woods Hole seawater ($\Delta = -1.82°$ C) to: (1) 50% seawater ($\Delta = -1.02°$ C), and (2) "fresh water" ($\Delta = -0.02°$ C) (Garrey, 1905, Table IV). After periods of six hours in 50% S.W. and three hours in F.W., the worms increased in weight (by an unstated amount) and appeared "swollen." In light of results from the present study, it is evident that Garrey's stress solutions were in the lethal range for the animals. Forced to base his judgment on Garrey's limited data, Oglesby could only conclude that *Chaetopterus* showed "no" ability to regulate its volume during osmotic stress (Oglesby, 1969a, Table X, page 251). The volume-regulating ability of *Chaetopterus* is, in fact, quite comparable in magnitude and rate to some of the better-known volume-regulating nereids, such as *Nereis diversicolor* (Beadle, 1937) and *N. limnicola* (Oglesby, 1968). In contrast to these euryhaline species, however, *Chaetopterus* appears to have no (or minor) salt-regulating capabilities and is unable to tolerate salinities as low as 50% S.W.

The mechanism by which an isoosmotic polychaete, such as *Chaetopterus*, "regulates" its volume after transfer to lower salinities is not known. There are currently two hypotheses to explain the corrective elimination of fluid in such animals. The first suggests that there is an increased efflux of fluid via the nephridia. The motive force driving this efflux may be produced intrinsically (*i.e.*, as a result of increased activity of cilia lining the nephridial tubules) or

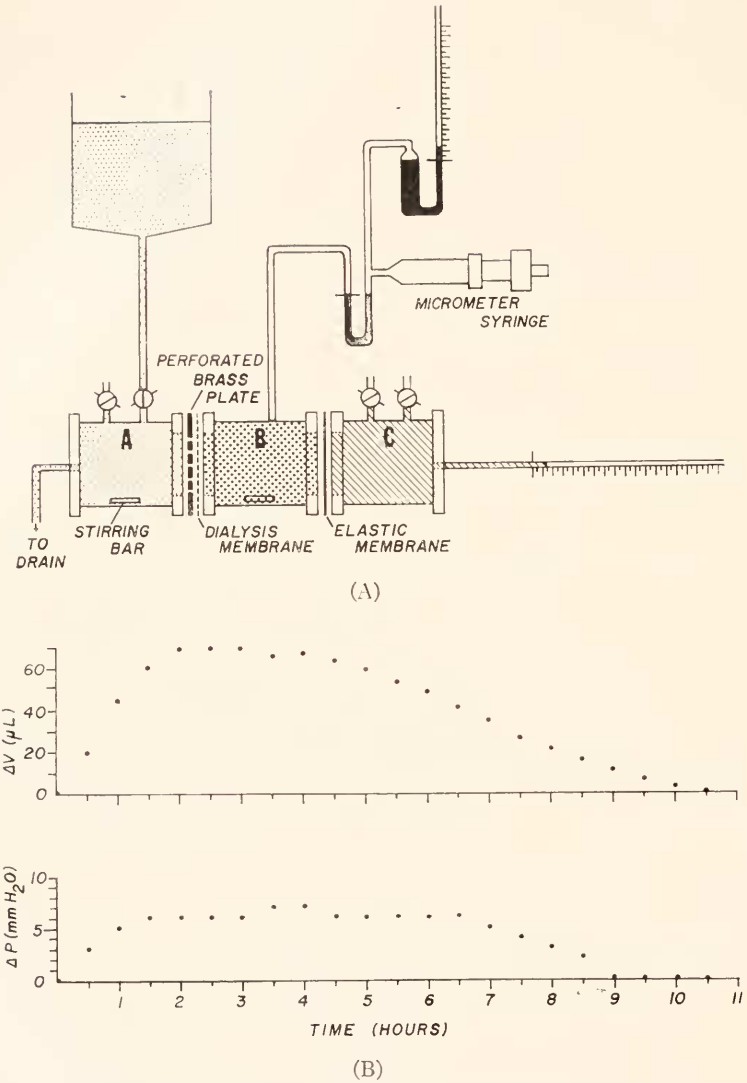

FIGURE 8. (A) "Mechanical engine" with properties of model. See text for details. (B) Time-course of volume and pressure changes in chamber B after osmotic gradient established at time 0.

extrinsically (*i.e.*, as a result of increased coelomic hydrostatic pressure caused by contraction of the body-wall musculature) (Beadle, 1937, 1943; Smith, 1970). A second hypothesis suggests that there is active transport of an ion species *outward* through the general integument. There presumably follows the passive diffusion outward of a counter-ion (maintaining electrical neutrality) and the passive diffusion outward of water (maintaining osmotic equilibrium). The total effect is the net transfer of isoosmotic saline from inside to outside (Prosser and

Brown, 1961). Although these proposed mechanisms differ markedly, they are not mutually exclusive. They are similar in presupposing that an expenditure of energy is required to produce the driving force necessary for fluid elimination. At present there is virtually no data to support or refute either of these hypotheses in detail, and even the studies investigating metabolic rates during volume regulation have failed to confirm or deny the existence of an energy requirement (Oglesby, 1969a).

One of the conceptual difficulties inherent in these theories relates to the negative-feedback character of the phenomenon, since data from many sources indicate that in good volume-regulators the amount of fluid efflux is such that the worms are returned rather precisely to their initial volumes (given sufficient time and sublethal stress). With reference to the active transport hypothesis, for example, there are many common systems where changes in salt concentration are known to elicit activity of membrane transport systems which act to correct the perturbation. When the salt concentration again approaches initial values, activity of the transport system is lowered to an appropriate level or stopped. Such monitoring of salt levels may be operative in polychaetes capable of both ion- and osmoregulation at low external salinities. With regard to isoosmotic volume-regulators, however, it would seem that there is a crucial component missing—namely, the internal salt concentration does not return to prestressed values during the following volume decrease. Therefore, a salt concentration "signal" to turn off the corrective effector activity (producing fluid efflux) does not appear to be present. In contrast, the hypothesis that a type of muscular stretch response produces hydrostatic back-pressure at least has the merit of suggesting how a mechanically linked "volumestat" servo-mechanism control might be achieved.

Nevertheless, it appears to us that the principle of parsimony has been all but ignored by the general assumption that the major route of fluid elimination is via the nephridial tubules. The apparent agreement on this point seems particularly puzzling since it is also generally acknowleged that the initial water entry occurs throughout the entire permeable integumental surface. No one has suggested, for example, that the initial rapid increase in volume results primarily from flow of water *inward* through the nephridial tubules. In addition, it is clear that active contraction of the body-wall musculature is not the only potential source of increased internal hydrostatic pressure. Apparently overlooked is the fact that the integumental membrane itself is an elastic or visco-elastic element and therefore must, at the very least, supplement muscular contraction in producing increased hydrostatic pressure.

We have therefore attempted to get some estimate of the magnitude of fluid efflux through the surface membrane of a hypothetical model "worm" in which the sole driving force is generated by stretched elastic or visco-elastic elements in the surface membrane. To do this we draw upon some of the concepts of nonequilibrium thermodynamics and make the following assumptions: (1) for ease in calculation, we model our hypothetical "worm" as a cylinder of uniform cross section of radius r and an unstressed volume V_0, for which we can neglect end-effects; (2) we assume that the bounding ("integumental") membrane of the model worm has *no* gross discontinuities (representing, for example, open metanephridial tubules); (3) we assume that the membrane possesses elastic properties

which can be adequately represented by Hooke's Law:

$$\Delta P = -G(r - r_0)$$

where ΔP = the change in pressure across the membrane, G = a constant, and the subscript zero denotes the value in the unstressed state; (4) we assume the membrane to be more permeable to water than to solutes; (5) we assume the membrane is not completely impermeable to solutes; (6) we assume that the permeability inward is equal to, or greater than, the permeability outward; (7) we assume that order-of-magnitude estimates for the values for permeability, change in radius, change in internal salt concentration, response time, *etc.* are identical with those derived from the experiments with *Chaetopterus* reported above; and (8) we assume *no* contribution to the system from contracting elements or active transport processes.

Using the basic equations of Kedem and Katchalsky (1958), the volume and salt fluxes across the membrane are respectively:

$$j = L_p(\Delta P - \sigma RT\Delta C) \tag{1}$$

$$\dot{n} = \omega RT\Delta C + (1 - \sigma)cj \tag{2}$$

where L_p is the filtration coefficient, σ is the reflection coefficient, ω is the solute mobility, R is the gas constant, T is the temperature, and ΔP, $\Delta C = C^0 - C^i$, and $C = \frac{1}{2}(C^0 + C^i)$ are, respectively, the differences in hydrostatic pressure, salt concentration, and the mean salt concentration across the membrane. Since L_p and ω may take on different values for inflow and outflow across the integument, we write

$$\left(\frac{L_p(t)}{\omega(t)}\right) = \left(\frac{L(0)}{\omega(0)}\right)[1 + \beta U(t - t^*)]$$

where $\beta \left(= \frac{L_p{}^{out} - L_p{}^{in}}{L_p{}^{in}}\right)$, is a constant, U(t) is the unit step function, and t* is the time at which the flow is reversed. Substituting these into (1) and (2), and making the change of variables (Crank, 1956)

$$I(t) = \int_0^t L_p(\tau)d\tau$$

The temporal changes of the radius of the cylinder and the number of moles of salt therein, N^i are given by

$$\frac{dR'}{d\hat{t}} = -\hat{G}R' - [\hat{C} - (1 + N')(1 + R')^{-2}]$$

$$\frac{1}{(1 + R')}\frac{dN'}{d\hat{t}} = \hat{\omega}[\hat{C} - (1 + N')(1 + R')^{-2}] + (1 - \sigma)[\hat{C} + (1 + N')(1 + R')^{-2}]\frac{dR'}{d\hat{t}}$$

where we have introduced the following scaled variables

$$R' = \left(\frac{r}{r_0} - 1\right), \quad N' = \left(\frac{N^i}{N_0{}^i} - 1\right), \quad \hat{C} = \frac{C^0}{C_0{}^i},$$

$$\hat{I} = \frac{I\sigma RTC_0{}^i}{r_0}, \quad \hat{G} = \frac{Gr_0}{\sigma RTC_0{}^i},$$

$$\hat{\omega} = \frac{2\,\omega(0)}{L_p(0)}\frac{1}{\sigma C_0{}^i}$$

Finding solutions to the above set of nonlinear equations would be a difficult task. However, observations made on *Chaetopterus* indicate that R′ and N′ do not exceed order 10^{-1}. Therefore, we may linearize the above, which gives

$$\frac{dR'}{d\hat{I}} = -(\hat{G} + 2)R' + N' + (1 - \hat{C}) \tag{3}$$

$$\frac{dN'}{d\hat{I}} = \hat{\omega}[2R' - N'] - (1 - \hat{C})\hat{\omega} + (1 - \sigma)(1 + \hat{C})\frac{dR'}{d\hat{I}} \tag{4}$$

Using Laplace transformations, it is a simple matter to find the two characteristic frequencies for (3) and (4) and the solution to our problem. We obtain

$$R'/(1 - \hat{C}) = e^{\lambda_2\hat{I}} \sinh\lambda_1\hat{I}/\lambda_1 \tag{5}$$

where

$$\lambda_1 = \tfrac{1}{2}\{[\hat{\omega} - (1 + \hat{C})(1 - \sigma) + \hat{G} + 2]^2 - 4\hat{\omega}\hat{G}\}^{\frac{1}{2}} > 0$$

$$\lambda_2 = -\tfrac{1}{2}[\hat{\omega} - (1 + \hat{C})(1 - \sigma) + \hat{G} + 2] < 0.$$

Clearly if $\hat{\omega}\hat{G} > 0$, R′ has the appropriate behavior, since I is a monotone increasing function of time. If we require that both the initial slope and maximum of equation (5) correspond to values obtained from the stress experiments on *Chaetopterus* (Fig. 5), we find

$$L_p(0)\frac{\sigma RTC_0{}^i}{r_0} \cong 8 \times 10^{-3}/\text{min.}$$

$$\hat{I} = (8 \times 10^{-3}/\text{min.})t\left[1 + \begin{cases} 0, & t \le t^* \\ \beta(1 - t^*/t), & t > t^* \end{cases}\right]$$

with λ_1, λ_2 in the range $.156 \le \lambda_1 \le .825$, $-1.04 \ge \lambda_2 \ge -1.25$. Figure 7 shows a comparison of the experimentally observed volume changes to those predicted on the basis of the calculations from our model. The curves on graphs 1, 2, and 3 were generated on the assumption that there exists a single L_p and β for the integument, and that our best estimate of these is derived from the mean value from all three experiments. The predicted curves on graphs 4, 5, and 6 were generated on the assumption that the different salinity stresses (and hence the degree of stretching of the membrane) influenced the L_p and β, and that our best estimate of these values is derived from the mean of each individual experiment.

In either case the graphs show remarkably good agreement between the calculated and the observed curves, consideration being given to the approximations in the former and the variability in the latter. We have also examined the case for a symmetrical system ($\beta = 0$), and find that it shows a similar response curve. As β is decreased from zero, the decay time from the state of maximal strain (to zero strain) is increased.

Using our calculations we can make an estimate of the values of the material constants σ, $\hat{G}$, and $\hat{\omega}$ ($\sigma \leq 0.05$, $\hat{G} \simeq 1$, and $\hat{\omega} \simeq 0.5$). Transforming back to unscaled variables, we have $L_p(0) \simeq 0.020$ cm(atm·min)$^{-1}$ $G \simeq 0.52$ atm, $\omega(0) \simeq 8.15 \times 10^{-8}$ moles (atm·min·cm^2)$^{-1}$. Since R'_{max} is of order 5×10^{-2}, we have ΔP_{max} of order 1×10^{-2} atm, which corresponds to a required maximum internal hydrostatic pressure of 7.6 mm of Hg, clearly within the range of measured coelomic pressures of polychaetes (Chapman and Newell, 1947; Prosser and Brown, 1961; Wells, 1945 and 1961). The small σ and $\omega(0)$ obtained are characteristic of a somewhat unselective membrane through which there is little solute flow at constant volume, which is consistent with the characteristics postulated for the integument of *Chaetopterus*.

The observed volume-regulation can be explained by the following mechanism. Initially, solvent flows into the worm as a result of the osmotic pressure difference across the membrane. (At the same time there is likely to be a small loss of salt to the exterior so that correctly the net volume increase equals volume H_2O inward minus volume salt outward. The initial water influx appears to be so rapid, however, that isoosmotic conditions obtain before any great volume of salts is lost and therefore it can be largely neglected.) The kinetic energy of water entry is transferred to the integument as a result of stretching the elastic elements. When the initial osmotic driving force is equalized (*i,e.*, isoosmotic conditions are attained) the energy stored in the distended membrane, via increased hydrostatic pressure, forces both salts and water back out through the membrane into the surrounding bath. The asymmetric nature of volume-regulation curves can be explained by the assumptions that although the fluid initially entering the animal (osmotically) is virtually pure water, the fluid leaving the animal (hydrostatically) must be isoosmotic with the body fluids, since preferential loss of water would set up an osmotic gradient in the opposite direction again. The rate of fluid (volume) loss will be determined by: (1) the hydrostatic pressure, and (2) the permeability of theintegument to both water and salt. Since the integument is less permeable to salts, the rate of efflux of isoosmotic saline will be decreased by this slower-moving component. The homeostatic nature of the mechanism is obvious. When the worm has returned to its original volume, the integument is no longer stretched—the energy stored in the elastic element having been completely dissipated in doing the work of expelling the fluid.

Such a mechanism requires no direct input of metabolic energy to the corrective process. It appeared possible to us, therefore, to construct a "mechanical engine" with (at least most of) the assumed properties of the system. Such a device, made up of 3 contiguous lucite chambers, is shown in Figure 8A. For practical purposes it was necessary to separate the elastic portion of the "integument" from the permeable, and thus the configuration of the mechanical engine was different than that of our theoretical model system (assumption #1,

above). However, the properties of the mechanical engine conformed closely to those outlined for our theoretical model in assumptions 2, 3, 4, 5, and 8, with the dialysis membrane being symmetrical ($\beta = 0$)—an alternative possibility in assumption 6. Assumption 7 was clearly impossible to achieve and thus the data are expressed in absolute units, without direct comparison to those derived from *Chaetopterus*. In practice a solute (sucrose) concentration difference of 0.1–0.5 M was initially established across the dialysis membrane, with chamber B (the "worm") hyperosmotic to chamber A (the "external medium"). The changes in pressure and volume in chamber B could be simultaneously monitored, with chamber C serving as a liquid-filled compensation chamber leading to the volume capillary. An example of the type of data obtained from the apparatus is shown in Figure 8B. The general shapes of the curves conform to the predicted behavior and show that such a completely mechanical model system is feasible.

Several comments are in order concerning the proposed mechanical "volumestat" model. First, such a system will only completely return to its initial volume if the membrane acts as a pure Hooke elastic element. We have performed the analysis assuming the integument to have both viscous and elastic properties, and thus to act as a Maxwell element

$$\Delta P = -G\left[(r - r_0) - \lambda \int_0^t e^{-\lambda(t-\tau)}(r - r_0)d\tau\right]$$

Understandably, the resulting curves vary, depending on the proportion of elastic to viscous properties. As G approaches zero (pure viscosity) there is swelling but no return. As λ approaches zero (pure elasticity) there is swelling followed by complete return. When both G and λ are greater than zero, there is swelling and partial return, depending on the specific values of the constants.

Secondly, we are fully cognizant that the integument of *Chaetopterus* is perhaps uniquely thin and distensible among those of the larger polychaetes. Previous histological studies, for example, have shown that *Chaetopterus* has, over large areas of its body, as few as two cell layers separating the coelomic fluid from the outer medium (Bonhomme, 1943; Krekel, 1920; Meissner, 1935; Nicol, 1952; Trojan, 1913). In addition, a recent electron-microscope survey study showed that *Chaetopterus* is exceptional in lacking a highly ordered fibrous "cuticle" associated with its integumental epithelium (Storch and Welsch, 1970). The thinness of the membrane, together with the apparent absence of cuticular fibers (a viscous element), is probably crucial to the remarkable agreement between our almost assuredly too simplistic theory and the actual experimental results.

Thirdly, we do not propose that our mechanical "volumestat" model is the whole answer in isoosmotic volume-regulating worms—indeed, it cannot be in those animals (possibly a majority) where viscous integumental elements predominate. In addition, it would seem unlikely that the evolutionary process would yield only a single mechanism for volume-regulation in polychaetes, or possibly for even a single species.

Nevertheless, it is clear that in any worm having a permeable outer integument which is even partially elastic, a saline efflux as outlined above will result. It may prove profitable to take this particular aspect into consideration in future investigations into the problem of volume-regulation.

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Summary

1. In *Chaetopterus* gradually adapted to lowered salinities, coelomic fluid osmolarity and sodium and chloride concentrations conform to ambient seawater at salinities from 1100 to 630 mOsm.

2. Experimental animals were unable to tolerate salinities below 630 mOsm for extended periods of time.

3. Worms transferred directly to hypoosmotic stress solutions down to 681.8 mOsm gained weight (volume) rapidly, reaching a maximum in approximately 1.5 hours. At this point, the coelomic fluid was isoosmotic with the external medium. After 1.5 hours, there followed a decrease in volume, which as shown in one experiment, culminated in a return to initial volume values.

4. Transfers back to full-strength seawater indicate that salt efflux as well as water influx occurred.

5. It is concluded that *Chaetopterus* is a volume-regulating osmoconformer over its viable range of salinities.

6. Current theories of the mechanism of isoosmotic volume-regulation are discussed and a mechanically linked "volumestat" model is proposed.

7. The behavior of the model system is mathematically analyzed utilizing the concepts of non-equilibrium thermodynamics.

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